Biocompatibility, bactericidal activity and cytotoxicity studies of carbon nanotubes

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Abstract

Our research interests are focused on the study of biocompatibility, bactericidal activity and cytotoxicity of biomaterials, coatings and nanoparticles. The studies we are carrying out are aimed to develop new assay procedures that allow predict the impact of these materials on the environment and human health.

The ongoing research is directed to make progress in the field of nanotechnology, taking carbon nanotubes (CNTs) as starting point. CNTs are becoming more widely used due to their physical and chemical properties, and their number of applications in a wide range of components and devices (such as composite structural materials, sensors, field emission displays, hydrogen storage materials, tips for scanning probe microscopy and semiconductor devices) is constantly increasing. In the biomedical field, CNTs are being extensively explored for delivery of therapeutic agents, diagnosis of diseases and regenerative materials [1]. This increasing use of CNTs requires more attention to nanotoxicology research, since human exposure to them is inevitable, thus the main objective of this study is to determine the toxicity of CNTs for the environment and human health [2-4].
IK4-TEKNIKER has a huge experience carrying out toxicity of chemical compounds using OECD standard protocols. Our laboratory has recently acquired new equipment to develop in vitro methods for toxicity assessment of nanoparticles, and new test protocols are being implemented to study biocompatibility, bactericidal activity and cytotoxicity of CNTs.

The first and one of the most important issues that our study is facing is to find an appropriate method to disperse CNTs in water, the toxicity tests media. Given their hydrophobic nature and tendency to aggregate, CNTs are inadequately soluble or dispersible in most of the common organic and inorganic solvents [5,6]. In this work we start from a previous research carried out in IK4-TEKNIKER, in which two CNTs trademarks have been characterized and tested to identify new solubilization or dispersion methods.

The toxicity study includes a series of three in vivo ecotoxicity assays: the inhibitory effect of potentially toxic substances on the light emission of Vibrio fischeri Luminescent bacteria test, Daphnia Magna Acute Immobilization Test and Alga (*Selenastrum Capricornutum*) Growth Inhibition Test.

The Dr. Lange's LUMIStox test of bioluminescent bacterium is a procedure in which Vibrio Fischeri bacterium produce light as a by-product of its cellular respiration. Toxic substances affect its cellular activity, resulting in a decreased rate of respiration and a corresponding decrease in the rate of luminescence. The test is based on the determination of the influence of chosen toxicant concentrations in the bacterium luminescence after a contact time of 15 minutes. This biotoxicity test measures an inhibitory effect as a function of the dilution of the

sample. So, the EC50-value (Effective Concentration causing 50% inhibition) is the commonly used result parameter.

In the Daphnia Magna Test a range of concentrations of the substance investigated exerts different degree of toxic effects on the swimming capability of Daphnia under otherwise identical testing conditions. Certain concentrations result in certain percentages of Daphnia being no longer capable of swimming at 24 and 48 hours, so the immobility percentage at 24-48 hours is determined for each dilution. The measure of inhibitive effect on Daphnia of the test sample is the EC50 value, determined graphically or by calculation. This indicates the concentration of the test sample at which 50% of the Daphnia used become incapable of swimming within the 24-48 hour test period.

Finally, the Alga Growth Inhibition Test is based on exponentially-growing cultures of selected green algae (Selenastrum Capricornutum) exposed to various concentrations of the test substance over several generations, under defined conditions during 72 hours. The test is performed in long cell test vials, with algae de-immobilized from algal beads, and optical density is the parameter to determine algal growth inhibition. The result parameter, EC_{50}, is the concentration of test substance which results in a 50% reduction in either growth or growth rate relative to the control.

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Figures

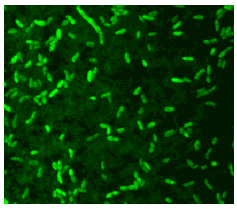
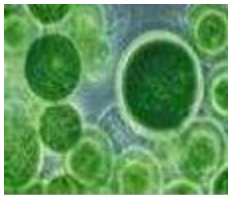
a)  b)  c) 

Figure 1. a) Vibrio Fischeri, b) Daphnia and c) Algae examples used for toxicity tests.

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